Gene Expression and Auditory Physiology Analyses to Determine the Roles of Connexin 30 and 43 in Age-Related Hearing Loss

Jennifer Pineros[1,3#], Xiaoxia Zhu[1,3], Bo Ding[2,3], Parveen Bazard[1,3], Robert D. Frisina[1,3*]
[1]Dept. of Medical Engineering, [2]Dept. of Communication Sciences & Disorders, [3]Global Center for Hearing & Speech Research, Univ. South Florida, Tampa, FL 33620

Introduction: Connexin proteins (Cx) are essential for formation of gap junctions, a basis for intercellular communication; and Cx gene expression exists in various cochlear regions. Mutations in Cx genes have been linked to human nonsyndromic deafness. However, no studies have been conducted on Cx's involvement in age-related hearing loss (ARHL). Also, the locations of Cx isoforms as matrix components in the cochlea are not identified. We identified locations and analyzed age-associated changes of *Cx30* and *Cx43* for future translational advances to treat ARHL.

Materials and Methods: CBA/CaJ mice (N=40) were classified into four groups according to age and degree of hearing loss, and an Affymetrix GeneChip equipped with transcriptional expression assessment for the mouse genome was used to measure *Cx* gene expression. The results were analyzed with one-way ANOVA and linear regression. To confirm our GeneChip results, RT-qPCR was conducted using the *in vitro* cell line SV-K1. Furthermore, to test gene expression with aging, an *in vivo* experiment with young adult (3 mon, n=4) and old (30 mon, n=4) cochlear samples were analyzed. Auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) were recorded to measure hearing changes.

Results and Discussion: All cochlear isoforms of the *Cx* family were analyzed using GeneChip, but only *Cx30* and *Cx43* exhibited significant expressional changes with age. Moreover, both genes showed a correlation with ARHL hearing threshold changes. The *in vitro* SV-K1 cell study detected only *Cx30* using RT-qPCR, suggesting *Cx30* but not *Cx43* is expressed in the stria vascularis (SV). Consistent with the young vs old *in vivo* study, a trend of down-regulation with age was apparent for *Cx 30* in the SV. Since other studies revealed *Cx43* expression in the cochlea, we conducted an *in vitro* organ of Corti (HEI-OC1) cell study, and both Cx30 and Cx43 showed expression. T-tests were performed and confirmed downregulation for both genes in the OC with aging.

Summary: Our study suggests a trend of downregulation of *Cx30* in the in the SV, as it was the only gene discovered in the SV from the *in vitro* study. Moreover, in the HEI-OC1, both *Cx30* and *Cx43* were found. Both genes showed a downregulation in the OC with aging in the young adult vs old experiment. Our long-term goal is to determine how aging affects the Cx family in different regions of the cochlea, and the impact on potential preventions and treatments for ARHL.

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Poster or Podium

Email: pinerosj@mail.usf.edu